

Pseudomonas moraviensis sp. nov. and *Pseudomonas vranovensis* sp. nov., soil bacteria isolated on nitroaromatic compounds, and emended description of *Pseudomonas asplenii*

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Two strains of Gram-negative bacteria isolated from soil by selective enrichment with nitroaromatics were subjected to a polyphasic taxonomic study. On the basis of 16S rRNA gene sequence analysis, the two strains were found to belong to the genus *Pseudomonas*, within the *Gammaproteobacteria*. Strain 1B4^T shared the highest sequence similarity with *Pseudomonas koreensis* DSM 16610^T (99.5%) and *Pseudomonas jessenii* CCM 4840^T (99.3%), and strain 2B2^T with *Pseudomonas asplenii* DSM 17133^T (98.9%), *Pseudomonas fuscovaginae* DSM 7231^T (98.9%) and *Pseudomonas putida* DSM 291^T (98.7%). On the basis of phylogenetic analysis, DNA–DNA hybridization and phenotype, including chemotaxonomic characteristics, two novel species, *Pseudomonas moraviensis* sp. nov. with the type strain 1B4^T (= CCM 7280^T = DSM 16007^T) and *Pseudomonas vranovensis* sp. nov. with the type strain 2B2^T (= CCM 7279^T = DSM 16006^T), are proposed. The description of *P. asplenii* was emended on the basis of additional data obtained in this study.

The genus *Pseudomonas sensu stricto* comprises almost 100 species with validly published names, at the time of writing (Anzai *et al.*, 2000; Hatayama *et al.*, 2005; Park *et al.*, 2005; Peix *et al.*, 2005; Romanenko *et al.*, 2005a, b; Clark *et al.*, 2006). Members of the genus are ubiquitous in soil, water and many other natural habitats (Palleroni, 1992). Due to their ability to utilize a broad spectrum of organic compounds (Stanier *et al.*, 1966), including environmental pollutants (Wackett, 2001), they belong to the biodegrading microflora (Andersen *et al.*, 2000; Fujii *et al.*, 2000; Pandey *et al.*, 2002; Ajithkumar *et al.*, 2003).

Abbreviations: 3-F-4-NP, 3-fluoro-4-nitrophenol; MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 2B2^T and 1B4^T are AY970951 and AY970952, respectively.

A table detailing characteristic peaks obtained by using intact cell MALDI-TOF MS and micrographs showing flagella staining of cells of *Pseudomonas moraviensis* 1B4^T and *Pseudomonas vranovensis* 2B2^T are available as supplementary material in IJSEM Online.

Strains 1B4^T and 2B2^T were isolated from samples of soil that originated from a place exposed to exhaust from motor vehicles in the village of Vranov, in the South Moravian region of the Czech Republic. Mineral medium (MM) described by Kotoučková *et al.* (2004), supplemented with 3-fluoro-4-nitrophenol (3-F-4-NP), 4-nitroguaiacol, 5-methyl-2-nitrophenol, 2-hydroxy-6-nitroaniline and 4,5-fluoro-2-nitrobenzoic acid, was used in the first step of enrichment and was followed by the use of 3-F-4-NP-supplemented medium in the second step of isolation. Ten millilitres of the medium supplemented with a mixture of nitroaromatic compounds was inoculated into 100 ml 3-F-4-NP-containing MM. Flasks were shaken on a rotary shaker at 28 °C. When the yellow colour of the medium had disappeared, samples were taken for repeated streaking on MM supplemented with 3-F-4-NP and solidified by addition of 1.5% (w/v) agar. Resulting single colonies were examined on nutrient agar (Oxoid CM3) at 28 °C.

Nutrient agar was used for routine cultivation of strains 1B4^T and 2B2^T and closely related type strains. The

following type strains were used: *Pseudomonas jessenii* CCM 4840^T (=DSM 17150^T=CIP 105274^T), *Pseudomonas koreensis* DSM 16610^T, *Pseudomonas putida* DSM 291^T (=CCM 7156^T), *Pseudomonas asplenii* DSM 17133^T and *Pseudomonas fuscovaginae* DSM 7231^T.

Cell size, morphology and colony appearance of strains 1B4^T and 2B2^T were assessed as described previously (Kotoučková *et al.*, 2004); the temperature range for growth and salt tolerance were determined on nutrient agar. Hydrolysis of Tween 80 and gelatin was tested according to Páčová & Kocur (1984). Additional biochemical properties were determined by using methods described by Smibert & Krieg (1994) and by using API 20NE, API 50 CH and Biolog GN MicroPlate systems. The commercial kits were used according to the manufacturers' instructions. Tests were read after 24 and 48 h with Biolog MicroStation System (MicroLog3 GN 4.20 database) for Biolog and visually for the API strips. Results read after 24 h are presented in Table 1 and the species description; related positive reactions of API 20NE and API 50 CH are given in parentheses.

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and purification of PCR products were carried out as described by Rainey *et al.* (1996). Purified PCR products were sequenced with a CEQ DTCS Quick Start kit (Beckman Coulter), according to the manufacturer's protocol. The CEQ 8000 Genetic Analysis System was used for electrophoresis of the sequence reaction products. The ae2 editor (Maidak *et al.*, 1999) was used to align the 16S rRNA gene sequences of strains 1B4^T and 2B2^T against those of representatives of the main bacterial lineages available from the public databases. Evolutionary distances were calculated using the method of Jukes & Cantor (1969). Phylogenetic dendrograms were constructed using the neighbour-joining algorithms (De Soete, 1983). Bootstrap analysis was used to evaluate the tree topology by performing 1000 resamplings (Felsenstein, 1993).

DNA for DNA–DNA hybridization experiments was isolated by using a French pressure cell and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA reassociation was performed under optimal conditions (2× SSC at 67 °C) and recorded using a model Cary 100 Bio UV/VIS-spectrophotometer (Varian) equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with an *in situ* temperature probe (Varian; Huß *et al.*, 1983). Automated ribotyping was carried out using the RiboPrinter microbial characterization system (Qualicon; DuPont) and *EcoRI* to generate restriction fragments.

The matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) technique was used for additional characterization of the isolates. Samples for MALDI-TOF MS analysis were prepared by suspending the cells in acetonitrile/water (1:1, v/v) and analysed immediately. Analyses were performed on a Reflex

IV instrument (Bruker); sDHB (90 % 2,5-dihydroxybenzoic acid and 10 % 2-hydroxy-5-methoxybenzoic acid; Bruker) was used as a MALDI matrix. Bacterial suspensions were mixed with sDHB matrix solution (40 mg ml⁻¹ in 20 % acetonitrile and 1 % trifluoroacetic acid) in a 1:4 (v/v) ratio. The mixture (0.6 µl) was pipetted on the MALDI target using the 'Dried-Droplet' sample preparation technique. Mass spectra measurements were performed in the linear positive mode. Mass spectra were calibrated externally using [M+H]⁺ and [M+2H]²⁺ molecular ion signals of lysozyme. At least seven consecutive spectra from three spots were measured for each sample. Spectra were evaluated with XTOF data-processing software, version 5.1.5 (Bruker). Peaks present in all measured spectra for a particular sample were taken into account for assessment of strain differences.

Cells for fatty acid analysis were harvested from 24 h cultures grown at 28 °C on trypticase soy broth (BBL) solidified by agar (Difco). Fatty acids were extracted and analysed as described by Čechová *et al.* (2004).

Phylogenetic analysis based on almost complete 16S rRNA gene sequences placed strains 1B4^T and 2B2^T in the genus *Pseudomonas sensu stricto*. The phylogenetic positions on the 16S rRNA gene tree are shown in Fig. 1. Strain 1B4^T formed a cluster with *P. koreensis* DSM 16610^T (99.5 % gene sequence similarity) and *P. jessenii* CCM 4840^T (99.3 %), and strain 2B2^T with *P. asplenii* DSM 17133^T (98.9 %), *P. fuscovaginae* DSM 7231^T (98.9 %) and *P. putida* DSM 291^T (98.7 %). The closest relatives of strains 1B4^T and 2B2^T (with 16S rRNA gene sequence similarity values above 98 %) were subjected to DNA–DNA hybridization. The DNA–DNA reassociation values were as follows: 40 % for strain 1B4^T and *P. koreensis* DSM 16610^T, 46 % for strain 1B4^T and *P. jessenii* CCM 4840^T, and 22, 12 and 43 %, respectively, between strain 2B2^T and *P. asplenii* DSM 17133^T, *P. fuscovaginae* DSM 7231^T and *P. putida* DSM 291^T. The DNA–DNA reassociation values of all closest relatives were clearly below 70 %, which is considered to be the threshold value for the delineation of genomic species (Wayne *et al.*, 1987).

As the DNA–DNA hybridization results of all the strains with the highest 16S rRNA gene sequence similarities uniformly showed values that were clearly below the species limit of 70 %, it is very unlikely that strains with lower similarity in the 16S rRNA gene sequence would reassociate with rates above the species limit. For the genus *Pseudomonas*, it was shown in previous studies that strains with 16S rRNA gene sequence similarities greater than 98 or even 99 % may belong to different species (Uchino *et al.*, 2001; Behrendt *et al.*, 2003; Hauser *et al.*, 2004). This is also true for groupings depicted in Fig. 1, among species highly related to strains 1B4^T and 2B2^T (Nishimori *et al.*, 2000; Dabboussi *et al.*, 2002; Kwon *et al.*, 2003). As early as 1999, Mohn *et al.* (1999) set the threshold for species delineation to 98 % similarity of the 16S rRNA gene in their description

Table 1. Characteristics that differentiate *Pseudomonas moraviensis* sp. nov. 1B4^T, *Pseudomonas vranovensis* sp. nov. 2B2^T and closely related *Pseudomonas* species

Strains: 1, *P. moraviensis* 1B4^T (=CCM 7280^T); 2, *P. vranovensis* 2B2^T (=CCM 7279^T); 3, *P. asplenii* DSM 17133^T; 4, *P. fuscovaginae* DSM 7231^T; 5, *P. putida* CCM 7156^T; 6, *P. jessenii* CCM 4840^T; 7, *P. koreensis* DSM 16610^T. +, Positive reaction; -, negative reaction, w, weakly positive reaction; ND, not determined. Values in parentheses were read after 48 h.

Characteristic*	1	2	3	4	5	6	7
Fluorescein (King B medium)†	+	-	+‡	+	+	+‡	+
Hydrolysis of:							
Starch†	-	-	-‡	+	-	-	-
Tween 80†	+	-	ND	+	-	-	+
Casein†	-	-	ND	-	-	+	-
Production of levan†	-	-	ND	-‡	-‡	+‡	-
Nitrate reduction ^a	-	+	-	-	-	+	-
Arginine dihydrolase ^a	- (+)	+	+	- (+)	+	-	+
Assimilation of:							
<i>N</i> -Acetylglucosamine ^a	+	-	-	-	-	w	+
Phenylacetate ^a	-	-	-	-	+	w (+)	-
L-Arabinose ^b	+	-	+	+	-	+	+
Ribose ^b	+	-	- (+)	- (w)	-	-	+
D-Xylose ^b	+	+	- (+)	-	-	-	-
Galactose ^b	+	-	- (+)	-	-	+	+
D-Mannose ^b	+	-	+	+	-	+	+
D-Mannitol ^b	+	-	+	+	-	+	+
Aesculin ^b	-	-	-	+	-	-	-
Trehalose ^b	+	-	+	+	-	-	-
D-Arabitol ^b	+	-	+	+	-	-	+
Utilization of:							
D-Arabitol ^c	+	-	+	-	-	+	+
Monomethyl succinate ^c	+	+	-	-	+	+	+
Formic acid ^c	-	+	+	-	+	+	+
D-Galacturonic acid ^c	-	-	w	+	+	-	-
D-Gluconic acid ^c	+	+	+	-	+	+	+
D-Glucuronic acid ^c	-	-	+	+	+	-	-
γ-Hydroxybutyric acid ^c	-	+	-	-	-	-	-
<i>p</i> -Hydroxyphenylacetic acid ^c	-	-	+	+	+	+	-
α-Ketovaleic acid ^c	w	-	-	-	w	w	-
DL-Lactic acid ^c	+	+	+	-	+	+	+
Malonic acid ^c	+	-	w	-	+	+	+
D-Saccharic acid ^c	+	-	+	+	+	+	+
Succinamic acid ^c	+	-	-	-	+	+	+
L-Alanine ^c	+	+	+	-	+	+	+
L-Asparagine ^c	+	+	-	+	+	+	+
Inosine ^c	+	-	+	+	+	-	+
Uridine ^c	+	-	w	-	-	-	+
2-Aminoethanol ^c	+	+	-	-	+	+	+

*Designated characteristics were determined with: a, API 20NE; b, API 50 CH; or c, Biolog GN.

†Conventional tests according to Smibert & Krieg (1994) and Páčová & Kocur (1984).

‡Data from Ark & Tompkins (1946), Miyajima *et al.* (1983), Verhille *et al.* (1999), Sikorski *et al.* (2001) and Dabboussi *et al.* (1999).

of *Pseudomonas multiresinivorans*, *Pseudomonas vancouverensis* and *Pseudomonas abietaniphila*.

Pseudomonas oryzihabitans IAM 1568^T was not included in the cluster of closely related strains of 2B2^T, although it

appears to be closely related [16S rRNA gene similarity value of strain 2B2^T with the available 16S rRNA gene sequence (GenBank accession no. D84004) is above 98 %]. The results of repeated 16S rRNA gene sequence analyses, performed at the DSMZ and at IAM, show just 95·8 %

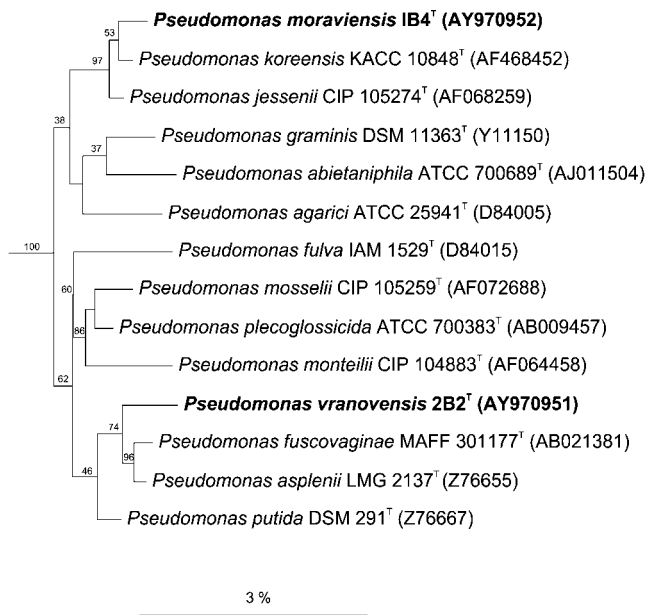


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence analysis showing the phylogenetic position of *P. moraviensis* sp. nov. 1B4^T and *P. vranovensis* sp. nov. 2B2^T. *Escherichia coli* was used to root the dendrogram (not shown). Bootstrap values (expressed as percentages) are given at branching points (1000 resamplings). Bar, 3 nucleotide substitutions per 100 nucleotides.

sequence similarity of *P. oryzihabitans* IAM 1568^T (GenBank accession no. AM262973) with the sequence deposited under accession number D84004. The sequence of *P. oryzihabitans* IAM 1568^T (AM262973) is identical with that of *P. oryzihabitans* DSM 6835^T (not submitted), so we believe that the sequence of *P. oryzihabitans* deposited under accession number D84004 is wrong. Apart from sequence analysis, no further tests were done. Interestingly, the sequence from *Pseudomonas oleovorans* IAM 1508^T (GenBank accession no. D84018) is almost identical to the sequence we obtained for *P. oryzihabitans* IAM 1568^T (99.6%), so it is possible that sequences D84004 and D84018 (Anzai *et al.*, 2000) were confused. The 16S rRNA gene similarity value of strain 2B2^T and *P. oryzihabitans* IAM 1568^T (AM262973) was 95.2%.

The closely related species were subjected to phenotypic characterization (Table 1). As the description of *P. asplenii*

(Ark & Tompkins, 1946; Savulescu, 1947) included only a few of the features needed for the present study, the type strain, DSM 17133^T (=ATCC 23835^T=NCPBB 1947^T=ICMP 3944^T), and a second strain, DSM 50254, were studied in detail, resulting in an emended description of the species. MALDI-TOF MS intact cell profile data are given as additional differentiation characteristics for strain 1B4^T, its closest relatives and strain 2B2^T (see Supplementary Table S1 in IJSEM Online).

Strain 1B4^T could be differentiated from *P. jessenii* CCM 4840^T by its ability to hydrolyse Tween 80, assimilate ribose, D-xylose, trehalose and D-arabitol, and utilize inosine and uridine, and by its inability to hydrolyse casein, produce levan or reduce nitrate. Assimilation of D-xylose and trehalose and the inability to utilize formic acid distinguish strain 1B4^T from *P. koreensis* DSM 16610^T (Table 1). MALDI-TOF MS intact cell profile is another characteristic useful for differentiating strain 1B4^T from *P. jessenii* CCM 4840^T and *P. koreensis* DSM 16610^T (see Supplementary Table S1 in IJSEM Online). Characteristics that distinguish strain 2B2^T from *P. asplenii* DSM 17133^T, *P. fuscovaginae* DSM 7231^T and *P. putida* CCM 7156^T are nitrate reduction, no fluorescein production on King B medium and utilization of γ -hydroxybutyric acid but not D-glucuronic acid, *p*-hydroxyphenylacetic acid or D-saccharic acid. Strains 1B4^T and 2B2^T differed, in addition to the characteristics given in Table 1, in the utilization of D-galactose, D-galactonic acid lactone and L-ornithine.

Moreover, the RiboPrint patterns of strains 1B4^T and 2B2^T (generated by using *EcoRI*) differed from those of the type strains of related *Pseudomonas* species (Fig. 2).

The cellular fatty acid content of strains 1B4^T and 2B2^T was very similar. The following fatty acids were detected for strain 1B4^T: 3-OH C_{10:0}, 2.6%; C_{12:0}, 2.1%; 2-OH C_{12:0}, 4.9%; 3-OH C_{12:0}, 4.1%; C_{14:0}, 0.4%; C_{16:1} ω 7c, 36.1%; C_{16:1} ω 5c, 0.1%; C_{16:0}, 28.8%; C_{17:0} iso, 0.2%; C_{17:0} cyclo, 2.4%; C_{17:0}, 0.2%; C_{18:1} ω 7c, 17.3%; C_{18:0}, 0.5%; and C_{19:0} cyclo ω 8c, 0.2%.

The cellular fatty acid content of strain 2B2^T was as follows: C_{10:0}, 0.1%; 3-OH C_{10:0}, 3.3%; C_{12:0}, 3.2%; 2-OH C_{12:0}, 4.1%; 3-OH C_{12:0}, 3.9%; C_{14:0}, 0.3%; C_{16:1} ω 7c, 27.0%; 2-OH C_{15:0} iso, 6.2%; C_{16:1} ω 5c, 0.1%; C_{16:0}, 27.0%; C_{17:0} iso, 0.2%; C_{17:1} ω 8c, 0.2%; C_{17:0} cyclo, 5.0%; C_{17:0},

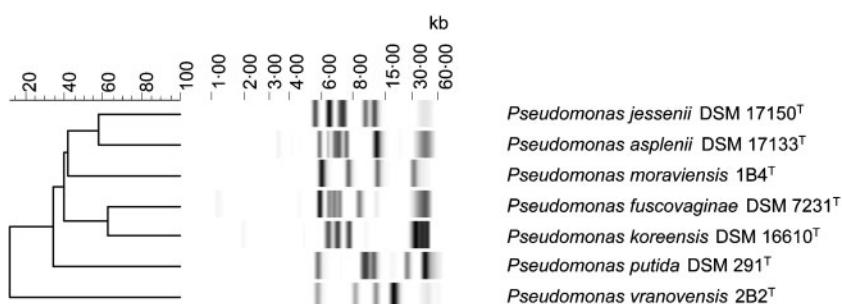


Fig. 2. RiboPrint patterns generated by using *EcoRI* for strains 1B4^T, 2B2^T and phylogenetically related type strains. The dendrogram of pattern similarity was calculated by using BioNumerics software (Applied Maths).

0.3 %; C_{18:1}ω7c, 18.5 %; C_{18:0}, 0.4 %; and C_{19:0} cyclo ω8c, 0.1 %.

Genetic and chemotaxonomic methods such as 16S rRNA gene analysis, DNA–DNA hybridization and whole-cell fatty acid analysis placed the two strains in the genus *Pseudomonas sensu stricto*. On the basis of genetic and phenotypic characteristics that distinguish strains 1B4^T and 2B2^T from their closest relatives, two novel species are proposed with the names *Pseudomonas moraviensis* sp. nov. and *Pseudomonas vranovensis* sp. nov.

Additional data generated for the type strain DSM 17133^T and strain DSM 50254 of *P. asplenii* for comparative purposes in this study resulted in an emended description of this species.

Description of *Pseudomonas moraviensis* sp. nov.

Pseudomonas moraviensis (mo.ra.vi'en.sis. N.L. fem. adj. *moraviensis* pertaining to Moravia, the region of the Czech Republic where strain 1B4^T was isolated).

Cells are Gram-negative, non-spore-forming rods, 0.6–1.3 × 2–5 μm, occurring singly or in pairs and motile by polar flagella (see Supplementary Fig. S1a in IJSEM Online). Cells form long rods (5–10 μm) in liquid medium (nutrient broth). Colonies on nutrient agar are circular, smooth and 2–3 mm in diameter (after 24 h of cultivation). Fluorescein is produced on King B medium. Pyocyanine is not produced on King A medium. Growth occurs at 4–35 °C, with optimum growth at 28–35 °C. Oxidase-positive. Urease- and DNase-negative. Nitrate reduction is negative. Tween 80, gelatin and tyrosine are hydrolysed, but not aesculin or starch. Lecithinase and indole are not produced and levan is not formed. By API 20NE, β-galactosidase is not produced and fermentation of D-glucose and assimilation of adipate are negative. Caprate, malate and citrate are assimilated. The following carbohydrates are assimilated (API 50 CH): glycerol, L-arabinose, D-glucose, D-fructose, D-trehalose, D-xylose (weakly positive after 96 h), gluconate and 2-ketogluconate. Negative reactions (API 50 CH) were observed for erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-xyloside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, D-fucose, L-fucose, L-arabitol and 5-ketogluconate. The following compounds are utilized (Biolog system): Tween 40, Tween 80, L-arabinose, D-fructose, D-galactose, α-D-glucose, D-mannitol, D-mannose, D-psi-cose, D-trehalose, methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, propionic acid, quinic acid, succinic acid, bromosuccinic acid, L-alaninamide, D-alanine, L-alanyl glycine, L-aspartic

acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-pyroglytamic acid, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, glycerol and DL-α-glycerol phosphate. Negative reactions (Biolog) are observed with α-cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, adonitol, D-cellobiose, erythritol, L-fucose, gentiobiose, myo-inositol, α-D-lactose, lactulose, maltose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, sucrose, turanose, xylitol, itaconic acid, sebacic acid, glycyl L-aspartic acid, L-ornithine, L-phenylalanine, D-serine, thymidine, phenylethylamine, 2,3-butane-diol, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Other reactions determined by API 20NE, API 50 CH and Biolog systems are given in Table 1. The fatty acid pattern is characterized by the presence of hydroxy fatty acids and cyclopropane fatty acids.

The type strain, 1B4^T (= CCM 7280^T = DSM 16007^T), was isolated from soil.

Description of *Pseudomonas vranovensis* sp. nov.

Pseudomonas vranovensis (vra.no.ven'sis. N.L. fem. adj. *vranovensis* of/from Vranov, the name of a village in South Moravia, the place of soil origin, the source of isolation of strain 2B2^T).

Cells are Gram-negative, non-spore-forming rods, 1–1.5 × 2–4 μm, occurring typically in pairs and motile by polar flagella (see Supplementary Fig. S1b in IJSEM Online). Colonies on nutrient agar are circular, smooth, non-pigmented and 2–3 mm in diameter (after 24 h of cultivation). Production of fluorescein (King B medium) and pyocyanine (King A medium) is negative. Growth occurs at 4–35 °C, with optimum growth at 28–35 °C. Growth occurs in up to 5 % NaCl. Oxidase-positive. Urease- and DNase-negative. Nitrate is reduced to nitrite. Nitrite is not reduced. Tween 80, gelatin, aesculin and starch are not hydrolysed. Lecithinase is not produced and levan is not formed. Tyrosine is hydrolysed. Indole is not produced. By API 20NE, arginine dihydrolase is produced but not β-galactosidase, and fermentation of D-glucose and assimilation of adipate and phenylacetate are negative. Caprate, malate and citrate are assimilated. The following carbohydrates are assimilated (API 50 CH): glycerol, D-glucose, D-fructose and gluconate. Negative reactions were obtained for erythritol, D-arabinose, L-arabinose, L-xylose, adonitol, methyl β-xyloside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-xylose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized (Biolog system): Tween 80, D-fructose, α-D-glucose, methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, β-hydroxybutyric acid, α-ketoglutaric acid, propionic

acid, quinic acid, succinic acid, bromosuccinic acid, L-alaninamide, D-alanine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, putrescine and glycerol. Negative reactions (Biolog) were observed with α -cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-cellobiose, erythritol, L-fucose, D-galactose, gentiobiose, myo-inositol, α -D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, D-galactonic acid lactone, D-glucosaminic acid, itaconic acid, sebacic acid, glucuronamide, L-alanyl glycine, glycyL L-aspartic acid, glycyL L-glutamic acid, L-phenylalanine, thymidine, phenylethylamine, 2,3-butanediol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Other reactions determined by API 20NE, API 50 CH and Biolog systems are given in Table 1. The fatty acid pattern is characterized by the presence of hydroxy fatty acids and cyclopropane fatty acids.

The type strain, 2B2^T (= CCM 7279^T = DSM 16006^T), was isolated from soil.

Emended description of *Pseudomonas asplenii* (Ark and Tompkins 1946) Savulescu 1947

Has the following properties in addition to those given previously (Ark & Tompkins, 1946). Oxidase-positive. By API 20NE, indole and β -galactosidase are not produced, aesculin and gelatin are not hydrolysed and fermentation of D-glucose and assimilation of adipate are negative. Caprate, malate and citrate are assimilated. Assimilation (API 50 CH) is positive for glycerol, D-glucose, D-fructose and gluconate, and negative for erythritol, D-arabinose, L-xylose, adonitol, methyl β -xyloside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate and 5-ketogluconate. The following compounds are utilized (Biolog system): methyl pyruvate, *cis*-aconitic acid, citric acid, formic acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α -ketoglutaric acid, quinic acid, bromosuccinic acid, D-alanine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-proline, L-serine, DL-carnitine, γ -aminobutyric acid and urocanic acid. Negative reactions (Biolog system) are observed with α -cyclodextrin, dextrin, glycogen, Tween 40, *N*-acetyl-D-galactosamine, adonitol, D-cellobiose, erythritol, L-fucose, gentiobiose, myo-inositol, α -D-lactose, lactulose, maltose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, D-galactonic acid lactone, γ -hydroxybutyric acid, itaconic acid, α -ketobutyric acid, sebacic acid, glucuronamide, glycyL L-aspartic acid, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine,

phenylethylamine, putrescine, 2,3-butanediol, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Other reactions determined by API 20NE, API 50 CH and Biolog systems are given in Table 1.

The type strain is DSM 17133^T (= ATCC 23835^T = CIP 106710^T).

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